I. Amendments

Claim 20 has been amended to recite that each oligonucleotide probe set is a subset of a mixture comprising oligonucleotides of all possible sequences of a selected length. Support is found, for example, at page 9, lines 1-2, which states that "Preferably, the oligonucleotide probes are applied to templates as mixtures comprising oligonucleotides of all possible sequences of a predetermined length", and at page 16, lines 11-12, which states that these probes "may be grouped into mixtures, or subsets" having similar duplex stability.

The amendment also replaces the language "have substantially the same free energy of duplex formation" with "are from the same stringency class". Support is found at page 16, lines 14-16. The description of how a stringency class is determined finds support in the specification at page 17, line 32 to page 8, line 37.

New claim 32 finds support in original claim 26.

In several claims (20-22, 24, and 28), "oligonucleotides" is amended to "oligonucleotide probes" for clarity. Dependency has been changed in claims 27-28, in view of the cancellation of claim 26. The apparently redundant term "defines" has been deleted from claim 30.

No new matter is added by any of the amendments.

II. Drawings

Enclosed are copies of the formal drawings submitted in parent application 08/424,663, now U.S. Patent No. 5,750,341. Margins are corrected and characters clarified, as required in Form PTO 948, dated 7/6/99. Formal drawings in the present case will be submitted upon allowance.

III. Rejections under 35 U.S.C. §112, Second Paragraph

Claims 20-31 were rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. This rejection is respectfully traversed for the following reasons.

Clarification of Terms

The applicants wish to point out, firstly, that the Examiner has misconstrued the length range of the claimed oligonucleotide probes. The Examiner refers to this length range as "from 3 nucleotides to 30 nucleotides", with a reference to claim 25, at several points in the Office Action (page 2, last line; page 5, first line, and page 6, lines 4-5). This is in error. The length of the oligonucleotide probes, as recited in claims 20 and 26, is "up to 12 nucleotides" or "8 to 12 nucleotides", respectively. Claim 25, on the other hand, recites the further element of an initializing oligonucleotide, which is 20 to 30 nucleotides long. That the oligonucleotide probes and the initializing oligonucleotides are separate elements is clear from

Fig. 1 and the related description at page 6, line 23 to page 7, line 5. The probes are designated by reference numbers **30** and **31** (page 6, lines 29, 36), while the initializing oligonucleotides are designated by reference symbols $\mathbf{i}_1 \ldots \mathbf{i}_n$ (page 6, lines 25, 29, 31, 33, 35; page 7, lines 2 and 4).

The applicants also point out that a "perfectly matched duplex", which the Examiner considers unclear, is defined in the specification at page 5, lines 18-24.

Specific Objections

The Examiner specifically objected to the claim phrases "substantially the same free energy of duplex formation" (claim 20) and "the same stringency class" (claim 26).

Claim 20 has been amended to replace the language "have substantially the same free energy of duplex formation" with "are from the same stringency class". The amended claim recites that a stringency class is determined by "(a) ordering a set of oligonucleotides of all possible sequences of a given length according to the free energy of binding of each oligonucleotide to its complement, and (b) selecting a subset of consecutive oligonucleotides within this ordered set." This process is described in the paragraph bridging pages 17-18 of the specification, with reference to algorithms that may be used for such ordering, and is illustrated on page 18 for the set of all 6-mers. While the illustration points to stringency classes of ten consecutive oligonucleotides, stringency classes may contain up to several thousand oligonucleotides, as stated at lines 42-43.

The test for definiteness is whether those skilled in the art would understand the bounds of the claim when read in light of the specification (*e.g.*, *Miles Laboratories, Inc. v. Shandon Inc.*, 997 F2d 870, 27 USPQ2d 1123 (Fed. Cir. 1993), *cert. denied*, 510 U.S. 1100 (1994)). The amended claim, and the specification, clearly define how a "stringency class" is determined. One of skill in the art would be able to determine whether a given subset of oligonucleotides falls within this definition, e.g., by ordering the full set of oligonucleotides of that length according to duplex stability, as described in the specification, and comparing the ordered list to the given set of oligonucleotides.

In view of the foregoing, the applicants submit that the claims, as amended, comply with the requirements of 35 U.S.C. §112, second paragraph.

IV. Rejections under 35 U.S.C. §102(b) / 103(a)

Claims 20, 21, 26, 28 and 29 were rejected under 35 U.S.C. §102(b) as being anticipated, or, alternatively, under 35 U.S.C. §103(a) as being obvious over, Pease *et al.* (*PNAS USA* **91**:5022, 1994). These rejections are respectfully traversed for the following reasons.

A. The Invention

The applicant's invention, as embodied in claim 20, provides a kit for DNA sequence analysis, useful in the polynucleotide sequencing methods described in the application. The kit includes one or more sets

of oligonucleotide probes, where (i) each probe set contains at least 50 different-sequence, single-stranded oligonucleotides, (ii) the oligonucleotides have lengths up to 12 nucleotides, (iii) each probe set is a subset of a mixture comprising oligonucleotides of all possible sequences of a selected length, and (iv) in each set, the different-sequence, single-stranded oligonucleotides within that set are from the same stringency class.

The benefits of using sets of probes from the same stringency class are described at page 16, lines 11-22 of the specification. As noted at page 9, first paragraph, the probes are preferably applied to the polynucleotide template as mixtures comprising oligonucleotides of all possible sequences of a given length. Such a mixture can be quite complex; for example, the full set of 8-mers includes over 65,000 oligonucleotides. In such a mixture, individual probes may not be present at concentrations sufficient to drive hybridization at a reasonable rate, particularly for sequences having lower free energy of binding.

This problem can be addressed by employing groups of stringency classes. As described at page 16, lines 11-21, each set of oligonucleotide probes from a single stringency class may be separately combined with the target polynucleotide under conditions such that substantially only oligonucleotide probes complementary to the target polynucleotide form duplexes. That is, the stringency of the hybridization reaction can be tailored to the free energy of duplex formation of the stringency class being used, so that substantially only perfectly complementary oligonucleotide probes form duplexes. This reduces the possibility of mismatches and errors in sorting or sequencing.

B. The Prior Art

Pease *et al.* was discussed in the response filed May 12, 2000. Briefly, this reference describes preparation of probe matrices which contain either (i) a single sequence, (ii) two different sequences, or (iii) a complete set of all possible sequences of a given length. The first two instances clearly do not show or suggest the probe sets of the invention, each of which contains at least 50 different-sequence oligonucleotides.

The third instance, in which all possible sequences are present, does not suggest a subset of probe oligonucleotides, belonging to a single stringency class, as defined above. Nor is there any suggestion or motivation in the reference to provide subsets of oligonucleotides from a single stringency class. The advantages of using such subsets in the applicants' disclosed sequencing methods, also discussed above, would not be provided by a set of all possible sequences.

In view of the above, the applicant respectfully requests the Examiner to withdraw the rejections under 35 U.S.C. §102(b)/103.

V. Conclusion

In view of the foregoing, the applicant submits that the claims now pending are now in condition for

allowance. A Notice of Allowance is, therefore, respectfully requested.

If in the opinion of the Examiner a telephone conference would expedite the prosecution of the subject application, the Examiner is encouraged to call the undersigned at (650) 324-0880.

No fees are believed necessary with this communication. However, the Commissioner is hereby authorized and requested to charge any deficiency in fees herein, or credit any overpayment, to Deposit Account No. 04-0531.

Respectfully submitted,

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